Published in final edited form as:
J Nutr Biochem. 2012 June ; 23(6): 532–538. doi:10.1016/j.jnutbio.2011.02.008.

Estrogen Status Alters Tissue Distribution and Metabolism of Selenium in Female Rats,[1,2]

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Abstract

A reported association between estrogen and selenium status may be important in the regulation of selenium metabolism. In this study the effect of estrogen status on the metabolism of orally administered ^{75}Se-selenite and tissue selenium status was investigated. Female Sprague Dawley rats were bilaterally ovariectomized at 7 weeks of age and implanted with either a placebo pellet (OVX) or pellet containing estradiol (OVX+E2), or were sham operated (Sham). At 12 weeks of age, 60 μCi of ^{75}Se as selenite was orally administered to OVX and OVX+E2 rats. Blood and organs were collected 1, 3, 6, and 24h after dosing. Estrogen status was associated with time dependent differences in distribution of ^{75}Se in plasma, RBC, liver, heart, kidney, spleen, brain, and thymus and incorporation of ^{75}Se into plasma selenoprotein P (Sepp1) and glutathione peroxidase (GPx). Estrogen-treatment also significantly increased selenium concentration and GPx activity in plasma, liver, and brain, selenium concentration in RBC, and hepatic Sepp1 and GPx1 mRNA. These results suggest that estrogen status affects tissue distribution of selenium by modulating Sepp1 as this protein plays a central role in selenium transport.

Keywords

selenium; estrogen; selenoprotein P; glutathione peroxidase; ovariectomized rats

1. INTRODUCTION

A relationship between estrogen status and selenium metabolism has been suggested. Selenium concentrations in blood [1,2], liver [3,4], and brain [5] have been reported to differ in adult male and female mammals and blood glutathione peroxidase (GPx) activity declines during pregnancy in rats [6] and humans [7,8], suggesting that estrogen modulates selenium

[1]Presented in part at Experimental Biology 07, April 2007, Washington DC [Zhou, X., Smith, A.M., and Failla, M.L. Estrogen Status Alters Tissue Distribution of Oral Dose of ^{75}Se-Selenite and Liver mRNA Levels of SelP and GPx. FASEB J 2007; 21 (5): 696.4 (Abs).].

[2]Supported by OARDC Grant OHO00201 and NIHES02497 (measurement of plasma selenoprotein P concentration by laboratory of RF Burk at Vanderbilt University School of Medicine, Nashville, TN)

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metabolism. Blood selenium and GPx activity were positively correlated with estrogen concentrations during the rat estrous cycle [9] and the human menstrual cycle [10]. Also, estrogen administration significantly increased erythrocyte GPx activity in both premenopausal [11] and postmenopausal women [12,13]. 17β-estradiol administration increased the amount of GPx4 mRNA, but not GPx1 mRNA or GPx3 mRNA, in the bovine oviduct [14]. GPx mRNA expression in hepatoma H4IIE cells exposed to the dietary phytoestrogen daidzein was also increased by 40% [15], but not by the addition of 17β-estradiol to cultures of human endothelial cells [16].

Twenty-five human selenoproteins have been identified including multiple isoforms of GPx [17]. Selenoprotein P (Sepp1) has attracted much attention in light of its role in the transport of selenium from liver to other tissues [18,19]. Sepp1 synthesis is regulated by dietary selenium intake as inadequate selenium is associated with decreased plasma Sepp1 in rats [20] and humans [21] and significantly lower Sepp1 mRNA levels in rat liver and kidney [22]. The potential influence of estrogen status on Sepp1 expression and activity has not been previously examined.

Sex-based differences in the health effects of selenium have been reported in a number of epidemiologic studies [23]. Results from prospective human studies indicate that anticarcinogenic effects of selenium are generally greater in men than in women [24], suggesting endocrine influences on the health-promoting effects of selenium. Although previous research has focused on the effect of estrogen status or estrogen administration on selenium concentration and GPx activity in plasma and erythrocytes, more specific investigations of the impact of estrogen on distribution and metabolism of selenium in multiple tissues are limited. The purpose of this study was to investigate the effects of estrogen status on the apparent absorption, tissue distribution and incorporation of selenium into selenoproteins. The effect of estrogen status on selenium status also was determined by determining selenium concentrations and GPx activity in different tissues, hepatic levels of Sepp1 mRNA and GPx mRNA, and plasma Sepp1.

2. MATERIALS AND METHODS

2.1. Animals and diets

Female Sprague Dawley rats (Taconic, Germantown, NY) weighing 45–55 g were fed the casein-based AIN-93G diet containing 150 μg total Se/kg diet as sodium selenate (Research Diets, New Brunswick, NJ) beginning at weaning (3 weeks) and throughout the study. At 7 weeks of age, rats were randomly divided into three groups. The first two groups underwent ovariectomy with (OVX+E2, n=22) or without (OVX, n=22) estrogen replacement. The third group was sham operated (Sham, n=24) and designed for 6 rats to be killed on each day of the estrous cycle. Estrous cycles were determined by examining vaginal smears as described previously [9]. Rats in the OVX+E2 group were implanted with a subcutaneous pellet containing 1.5mg 17β-estradiol for release over 60 days (Innovative Research of America, Sarasota, FL). Rats in the OVX and Sham groups were subcutaneously implanted with a placebo pellet. The animals were housed in individual plastic cages in a room with controlled temperature (20–22°) and a 12h light-dark cycle with lights off from 1800 to 0600 h. Animals were fed between 0800 and 0900h. Because OVX+E2 rats had lower food intakes than the other groups, the OVX+E2 group was given free access to diet, and the OVX and Sham groups were pair-fed the mean intake of the OVX+E2 group during the previous day in order to maintain similar caloric and selenium intake among animals. Food intakes were recorded daily and body weights were recorded weekly. Animals were fasted overnight before sacrifice. All procedures involving animals were approved by the Institutional Animal Care and Use Committee of The Ohio State University.

2.2. Experimental design

The effect of estrogen on the metabolism of ^{75}Se-selenite was studied in the OVX+E2 and OVX groups because they were expected to have the higher and lower plasma concentrations of estrogen respectively, than those for the Sham group. At 12 weeks of age, the OVX and OVX+E2 groups were administered 60 μCi of ^{75}Se-sodium selenite with specific activity of 1,400 Ci/g (University of Missouri Research Reactor Facility, Columbia, MO) in 1.0 ml 150 mM NaCl by gavage. Four rats in each group were killed 1, 3, 6, and 24 h after ^{75}Se administration. Rats were anesthetized by brief exposure to carbon dioxide prior to terminal cardiac puncture for collection of blood into syringes containing 10 mg Na_2EDTA in 100 μl saline. Liver, kidney, heart, brain, lung, thymus, spleen, and the gastrointestinal tract (GI tract, including esophagus, stomach, duodenum, small intestine, large intestine, rectum, and the anus) and its contents were collected. Another set of rats from the OVX (n=6), OVX+E2 (n=6), and Sham (n=24) groups that did not receive ^{75}Se were also killed at 12 weeks of age to collect blood, liver, kidney, heart, and brain for determination of total selenium content, GPx activity, hepatic levels of GPx and Sepp1 mRNAs, and plasma Sepp1. All samples were stored at −80° before analysis.

2.3. Plasma 17β-estradiol and ceruloplasmin activity

Concentration of plasma 17β-estradiol was determined using a double-antibody estradiol ^{125}I radioimmunoassay kit (Diagnostic Products Corp., Los Angeles, CA). Because the copper containing protein ceruloplasmin has been shown to be upregulated by estrogen [25], ceruloplasmin enzyme activity served as a positive control for estrogen-mediated alteration of trace element metabolism. Ceruloplasmin activity was measured according to Schosinsky et al. [26] with *o*-dianisidine dihydrochloride as the substrate.

2.4. ^{75}Se in tissues, cytosol, and membrane

^{75}Se was determined in plasma, RBC, and organs with a Cobra II auto-gamma counter (Packard Instrument Company, Meriden, CT). As organs were not perfused during collection, contamination of erythrocytes and plasma was estimated via hemoglobin (Hb) concentrations and subtracted from the totals in organs. For organs in which the ^{75}Se activity differed between the OVX and OVX+E2 groups, the distribution of ^{75}Se in total membrane (organelle) and cytoplasmic compartments was determined. Organs were homogenized in 50 mM phosphate buffer (25% homogenate) containing 10% sucrose, pH 6.3, using a Brinkman Polytron homogenizer (Brinkman Instruments Co., Westbury, NY). An aliquot of the homogenate was centrifuged at 4° for 90 min at 110,000 × g (Ti 50 rotor, Beckman Model L7-65, Palo Alto, CA) and supernatant (cytosol) was removed to measure ^{75}Se activity. Activity was normalized by protein concentration determined by the bicinchoninic acid method (BCA protein assay kit, Pierce, Rockford, IL). ^{75}Se activity in the membrane fraction was estimated by subtracting activity in cytosol from the total activity in homogenate.

2.5. Determination of ^{75}Se in selenoproteins

The incorporation of ^{75}Se into selenoproteins was determined using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Proteins in plasma and cytosol were separated with 5% stacking and 12% resolving gels. 10 μl samples containing 50–100 μg protein was loaded to each well. Gels were sliced according to the known molecular weights of well characterized selenoproteins and the ^{75}Se activity in gel slices was measured by gamma ray spectrometry (Packard Cobra II Auto Gamma; Packard Instrument Company, Meriden, CT). This procedure provides a convenient method to examine the ^{75}Se distribution among selenoproteins without the use of antibodies, and has been used to identify selenoproteins with molecular weights from 12 kDa to 75 kDa [27]. Total

protein ^{75}Se was defined as the sum of ^{75}Se activity of every band with molecular weight between 12 kDa and 75 kDa. Here we investigated the distribution of ^{75}Se into Sepp1 and GPx by using this method.

2.6. Selenium Status

Selenium concentrations in diet, plasma, RBC, liver, kidney, heart, and brain were measured by the gas chromatography technique of McCarthy et al. [28] using an Agilent 6890 Series gas chromatograph with a 225 Durabond Megabor column and an electron capture detector maintained at the following temperatures: oven (column) 190°C, front inlet (injector) 220°C, and front detector 300°C. Nitrogen (~60 psi) and helium (~80 psi) were used as carrier gases. Data were integrated using an Agilent 6890 Series Integrator. GPx activity of plasma, RBC, liver, kidney, brain, and heart was determined by the coupled assay of Paglia and Valenine [29] using a Shimadzu UV-visible recording spectrophotometer (model UV160U). The activity was expressed as units per gram of protein (hemoglobin for RBC). One unit of activity is equivalent to 1 μmol NADPH oxidized per minute at 37°C. The protein concentration in plasma and cytosol was measured by the bicinchoninic acid method (Pierce, Rockford, IL) with the Shimadzu UV-visible recording spectrophotometer (model UV160U). The hemoglobin concentration of RBC was determined with the Shimadzu UV-visible recording spectrophotometer (model UV160U) using Drabkin's reagent. Plasma Sepp1 concentration was determined by a competitive radioimmunoassay using a modified method of Hill et al. [30].

2.7. Hepatic levels of Sepp1 mRNA and GPx1 mRNA

Total RNA was extracted from the liver of rats in the OVX (n=6) and OVX+E2 (n=6) groups using a RNAqueous-4PCR kit (Ambion, Austin, Texas). First stand cDNA was synthesized from 1 μg RNA samples using the SuperScript First-Strand Synthesis System for RT-PCR (Invitrogen, Carlsbad, CA). To quantify the cDNA of Sepp1 and GPx1 mRNA, quantitative real-time PCR analysis was performed in an Mx3000P QPCR® system (Stratagene, La Jolla, CA) using the published primers for Sepp1 [31], GPx1[32], and GAPDH [33]. Temperature cycling conditions were 95°C for 2 min, 45 cycles of 95°C for 30 s, and 60°C for 1 min. The amounts of GAPDH mRNA were used to normalize expression.

2.8. Statistical methods

Data were reported as means±SE. The Student t-test was used to detect the differences between OVX+E2 and OVX groups. Analysis of Variance (ANOVA) was utilized to determine the differences of the indices at different times after administering ^{75}Se, and to determine the differences of the indices among treatment groups in the non-radioactive study. Differences were considered significant at $P<0.05$. When significant difference among the means was indicated, Tukey's *post hoc* probability test was performed to determine which means differed significantly. All statistical analyses were performed using SPSS V18 (SPSS Inc., Chicago, IL).

3. RESULTS

3.1. Body weights

Body weights for the three experimental groups prior to the ovariectomy and sham operations at 7 weeks of age were not significantly different ($P > 0.05$, Fig. 1). Chronic administration of estrogen to ovariectomized rats (OVX+E2) decreased body weight gain during the first post-operative week. Rats in the OVX group had the most rapid increase in body weight, followed by the Sham group, and then the OVX+E2 group despite using a pair-feeding paradigm. At weeks 8–10, body weights of the OVX and Sham groups were not

significantly different, but significantly greater than that of the OVX+E2 group ($P < 0.05$). At weeks 11 and 12, body weight differed significantly among the three groups with OVX > Sham > OVX+E2 ($P < 0.05$).

3.2. Plasma 17β-estradiol concentrations and ceruloplasmin activity

Ovariectomy (OVX) significantly ($P < 0.05$) reduced plasma 17β-estradiol concentration, whereas estrogen replacement (OVX+E2) significantly ($P < 0.05$) increased plasma 17β-estradiol concentrations greater than that in sham operated rats (Fig. 2A). Plasma 17β-estradiol levels were significantly greater during proestrus than at other stages of the estrus cycle ($P<0.05$) in sham-operated rats, which is a typical pattern during the 4-day estrus cycle. Plasma ceruloplasmin activity was positively associated with plasma 17β-estradiol concentration (r=0.78, $P<0.01$) and significantly ($P<0.05$) greater in the OVX+E2 group than in the Sham and OVX groups (Fig. 2B). There were no differences in plasma ceruloplasmin activity between the OVX and the Sham groups except during the proestrus stage.

3.3. ^{75}Se activity in tissues

As expected, ^{75}Se activity in the GI tract decreased from 1h to 24h with less than 5% of administered dose remaining in the gut by 24h (Fig. 3A). Because the amount of administered selenium excreted from feces during the first 24h is known to be relatively small [34], our results suggest efficient absorption of ^{75}Se by all treatment groups. There were no significant differences in ^{75}Se activity in the GI tract between the OVX and OVX +E2 groups at any test time. There was a significant increase ($P<0.05$) of ^{75}Se activity in blood and most organs in both groups from 1h to 3h (Fig. 3). ^{75}Se activity differed ($P<0.05$) between the OVX+E2 and OVX groups in plasma at 1, 3, 6h (OVX>OVX+E2) (Fig. 3B), RBC at 1, 3 (OVX>OVX+E2), and 24h (OVX+E2>OVX) (Fig. 3C), liver at 1 (OVX>OVX +E2), 6h (OVX+E2>OVX) (Fig. 3D), heart at 1, 3h (OVX>OVX+E2) (Fig. 3E), kidney at 3h (OVX>OVX+E2) (Fig. 3F), spleen at 1h (OVX>OVX+E2, data not shown), thymus at all 4 times (OVX >OVX+E2) (Fig. 3G), and brain at 6h (OVX+E2>OVX) (Fig. 3H).

3.4. Distribution of ^{75}Se among subcellular fractions and selenoproteins

Within organs that differed in total ^{75}Se activity between the OVX and OVX+E2 groups, ^{75}Se activity in both cytosol (Table 1) and membrane fraction (data not shown) of these tissues also differed. The distribution of ^{75}Se between cytosol and the membrane fraction was not significantly affected by chronic estrogen treatment (data not shown). Estrogen treatment also did not affect the fraction of ^{75}Se incorporated into proteins and non-protein bound form in these organs (data not shown). ^{75}Se-GPx activity accounted for 31–38% of the total protein ^{75}Se activity in liver, 28%–31% in heart, and 24%–26% in kidney, spleen, thymus, and brain. In plasma, a greater percentage of ^{75}Se was present in Sepp1 in the OVX+E2 group than in plasma from the OVX group at 3, 6 and 24h (Fig. 4). ^{75}Se was not detected in plasma GPx at 1, 3, and 6h. However, a greater percentage ($P < 0.05$) of ^{75}Se was associated with plasma GPx at 24h in the OVX+E2 group (8.6%) than for the OVX group (6.5%).

3.5. Tissue selenium concentrations and GPx activity

Estrogen status affected selenium concentrations in plasma, RBC, liver, and brain (Table 2). Plasma selenium concentration was significantly ($P < 0.05$) greater in OVX+E2 than sham-operated rats and plasma selenium was significantly ($P <0.05$) lower in OVX group than sham-operated rats at all stages of estrus cycle. Plasma selenium concentrations in sham-operated rats were greatest during proestrus. Selenium concentrations in RBC, liver, and brain were significantly higher in OVX+E2 rats than sham-operated rats at all stages of the

estrus cycle, except proestrus. OVX rats had significantly lower selenium concentrations in RBC, liver, and brain compared to OVX+E2 and sham-operated rats in the proestrus stage. Selenium concentrations in kidney and heart were independent of plasma estrogen.

Estrogen status affected GPx activity in plasma, liver, and brain (Table 3). GPx activity was significantly ($P < 0.05$) greater in plasma of OVX+E2 rats compared to plasma of OVX rats and sham-operated rats during all estrous stages except proestrus. There were no significant differences in GPx activity in plasma and liver between OVX rats and sham-operated rats in all estrous stages except proestrus. OVX was associated with decreased GPx activity in brain, with significantly ($P < 0.05$) lower GPx activity than that of sham-operated rats in proestrus stage and OVX+E2 rats. OVX+E2 rats had greater GPx activity in brain than that of sham-operated rats in all estrous stages except for proestrus.

3.6. Plasma selenoprotein P concentration

No differences in plasma Sepp1 concentrations were observed among groups. Plasma Sepp1 concentrations (μg/ml, means ± SEM, n=6) were 22.8 ± 1.4, 26.1 ± 1.4, 22.1 ± 1.3, 27.4 ± 0.6, 21.7 ± 2.9, 23.4 ± 2.1 for groups of OVX+E2, OVX, diestrus, proestrus, estrous, and metestrus stages, respectively (Fig. 5).

3.7. Hepatic levels of Sepp1 and GPx1 mRNA

Hepatic Sepp1 mRNA levels were 88% greater in the OVX+E2 group compared to the OVX group (0.94 vs. 0.50, $P<0.05$) (Fig. 6). Likewise, the amount of GPX1 mRNA in liver of the OVX+E2 group was significantly ($P < 0.05$) greater than that in liver from the OVX group (2.8 vs. 2.2).

4. DISCUSSION

An ovariectomized rat model was used to investigate the effect of estrogen status on selenium metabolism. The results indicate that estrogen affected the tissue distribution and metabolism of an oral dose of ^{75}Se-selenite and increased total Se concentrations and GPx activity in plasma and tissues. Estrogen status for the three groups of rats was verified by measuring 17β-estradiol concentrations in plasma. Ovariectomy significantly decreased 17β-estradiol levels, whereas implantation with estrogen pellets elevated 17β estradiol to a concentration approximately 50% greater than that present during the proestrus phase of the estrus cycle in sham-operated rats (Fig. 2A). The results are also in agreement with previous studies in which estrogen treatment suppressed body weight gain [35,36] and increased plasma ceruloplasmin activity [25] (Fig. 2B). Since rats were pair-fed the same amount of selenium, OVX+E2 rats would have received relatively more selenium per unit body weight, suggesting that estrogen could regulate selenium metabolism via affecting the growth of animals.

The present study is the first to compare the impact of estrogen status on the apparent absorption and distribution of selenium among tissues, subcellular fractions, and selenoproteins. Although estrogen status did not affect selenium apparent absorption, it altered the uptake of ^{75}Se into tissues. A surprisingly quick uptake of ^{75}Se into RBCs (3h) and liver (1h) was observed in OVX rats (Fig. 3). The mechanism for this observation in RBCs and liver needs to be further elucidated. Estrogen also altered the distribution of ^{75}Se in the plasma selenoproteins, Sepp1 and GPx. A greater percentage of ^{75}Se was incorporated into plasma Sepp1 at 3, 6, and 24h in OVX+E2 group than in the OVX group (Fig. 4). Within plasma proteins, ^{75}Se was associated primarily with Sepp1 in plasma. These results agree with previous studies using a similar method in which 68% of ^{75}Se was deposited in Sepp1 when female rats were injected intraperitoneally with ^{75}Se-selenomethione [37]. Previous studies have demonstrated that ^{75}Se in plasma was distributed among Sepp1, GPx,

and other un-characterized low molecular weight selenium [38]. In the present study approximately 30% and 70% of plasma ^{75}Se was incorporated into Sepp1 and low molecular weight selenium, respectively, 1 h after gavage. This was followed by a rapid increase in ^{75}Se incorporation into Sepp1 with approximately 75% plasma ^{75}Se present in Sepp1 by 3h. Thereafter, ^{75}Se content in Sepp1 declined suggesting that Sepp1 delivered newly absorbed selenium to other organs, including kidney, the site of plasma GPx synthesis. Though ^{75}Se incorporation into Sepp1 was increased (Fig. 4) by estrogen treatment, plasma total ^{75}Se was decreased (Fig. 3B), suggesting estrogen may affect the partitioning of ^{75}Se among each component of plasma selenium, e.g. some components had increased ^{75}Se incorporation, while others had decreased ^{75}Se incorporation by estrogen treatment. ^{75}Se-GPx was not detected in plasma until 24h. This finding is in agreement with previous studies in which ^{75}Se-GPx was undectable in rat plasma during the first 6h after intravenous injection of ^{75}Se-selenite, whereas ^{75}Se-Sepp1 was detected at 1h [38].

The results of the present study also confirm that measures of selenium status in blood were affected by estrogen. Chronic estrogen treatment significantly ($P<0.05$) increased plasma GPx activity and RBC content of selenium. These results agree with those of the ^{75}Se tracer study, in which ^{75}Se activity was greater in plasma GPx (8.6% vs. 6.5% of the total protein ^{75}Se activity) and RBC of the OVX+E2 group at 24h (Fig. 3C). As selenium concentrations were measured after 5 weeks of treatment (from 7wk to 12wk), it is not surprising that effects of estrogen treatment on selenium concentrations (Table 2) were opposite that of distribution of ingested ^{75}Se in plasma, RBC, and liver at 1h (Fig 3). At later times, estrogen effects on ingested ^{75}Se distribution were more similar to that of the selenium concentrations. It would be interesting to follow up the ^{75}Se tracer study for a longer time, e.g. up to a few weeks to further determine the patterns of estrogen effects on ^{75}Se distribution. The effect of estrogen on selenium status in human studies has been inconsistent. Whereas some studies have reported that estrogen increased blood selenium and GPx activity in women [10–13], others have not observed an effect of estrogen on selenium status [39–41]. These differences among human studies probably stem from differences in subject age, health status, selenium intake, and lack of control of estrogen status.

Earlier studies reported that hepatic GPx activity is greater in females than in males [1,3,4], suggesting hormonal influences on selenium utilization. In this study both hepatic selenium concentration (Table 2) and GPx activity (Table 3) were greater when estrogen was available in the OVX+E2 group and at the proestrus stage. The results also show that estrogen facilitated post-absorptive ^{75}Se accumulation in liver during the initial 6h and ^{75}Se efflux from liver to other tissues after 6h. Total ^{75}Se in liver was greater in OVX than in OVX+E2 rats at 1h (13.1% vs.3.9% of dose, Fig. 3D), but ^{75}Se activity increased in the OVX+E2 group markedly from 1 to 6h and was greater than in the OVX group (18.0% dose vs. 10.9% dose). Because the liver is the central organ for selenium metabolism, these effects of estrogen on hepatic selenium status have important implications for whole body selenium metabolism. More specifically, real-time RT-PCR showed that estrogen upregulated hepatic mRNA levels of both Sepp1 and GPx1 (Fig. 6). Estrogen-mediated increases in hepatic GPx1 mRNA may increase GPx1 synthesis, and thus greater hepatic GPx activity in OVX+E2 rats. Estrogen treatment had much greater effect on hepatic Sepp1 mRNA levels (50%, Fig. 6) than on plasma ^{75}Se-Sepp1 (10%, Fig. 4) and Sepp1 concentration (no difference, Fig. 5), these differences may due to isoforms of Sepp1 existing in plasma [42], or a posttranscriptional regulation (turnover) of Sepp1. Similarly, estrogen treatment did not have the same extent of effects on hepatic GPx1 mRNA levels (30%, Fig. 6) as those of GPx activity in blood and tissues (Table 3) due to the turnover of GPx. The impact of estrogen on ^{75}Se distribution and hepatic levels of Sepp1 mRNA and GPx mRNA was not investigated in the Sham group, thus limiting our insights into the

effects of estrogen fluctuations within a more physiological range. In addition, only two forms of inorganic selenium (sodium selenate and ^{75}Se-sodium selenite) were investigated in this study. Since metabolism of selenium differs greatly by their forms, how estrogen affects metabolism of other forms of selenium, especially those commonly present in foods, needs to be determined in future studies.

Sepp1 synthesis has been shown to be regulated by the selenium supply posttranscriptionally [43] and by cytokines and growth factors transcriptionally [44, 45]. The regulation of Sepp1 synthesis by estrogen has not been reported previously. Estrogen is known to modulate the expression of target genes containing estrogen response elements (ERE) in their promoter region [46]. Although the Sepp1 gene lacks a complete ERE, it contains ERE half-sites (5′-AGGTCA-3′) in the promoter regions. The importance of the estrogen receptor (ER) binding as a monomer to an ERE half-site in some genes remains controversial [47]. The possibility that estrogen regulates expression of Sepp1 by binding of an activated ER to an ERE-half site warrants further investigation. In the absence of an ERE, estrogen may regulate gene expression by a non-classical pathway. Estrogen binding to ER is known to activate transcription factors such as AP1, Sp1 and NF-kB. These proteins bind to cognate recognition sequences in DNA directly and influence the transcription of genes [48, 49]. The Sepp1 promoter contains two binding sites for AP1, and one binding site for Sp1, but no binding site for NF-kB [44,50]. Thus, estrogen may regulate transcription of GPx1 and Sepp1 through binding of AP1 and Sp1 to cognate sites. Additional studies are needed to determine if estrogen may modulate expression of Sepp1 gene expression by direct binding of ER to the ERE-half site or perhaps via activation of transcription factors such as AP1 and Sp1, or by a combination of both pathways.

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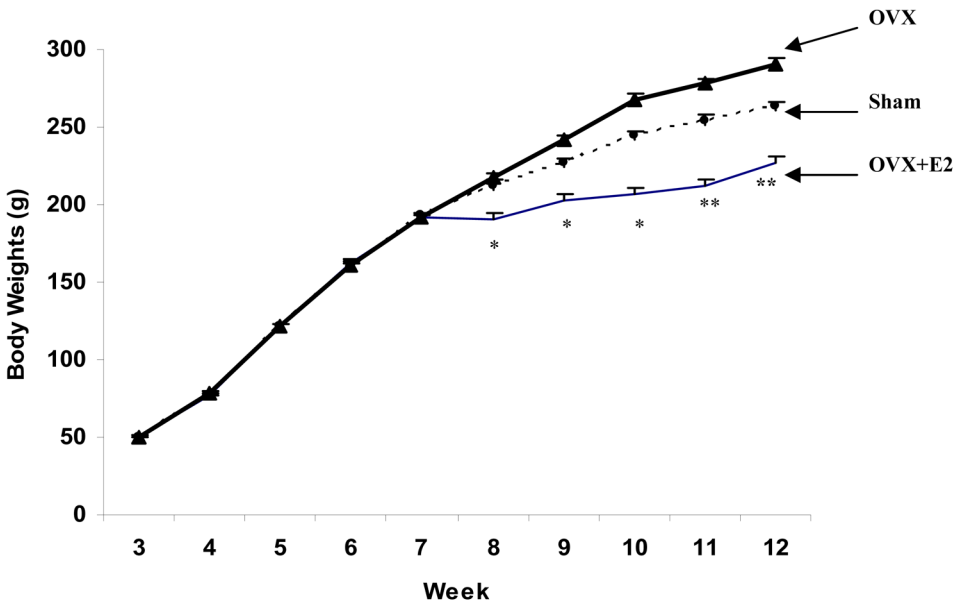

Fig.1.

Body weights of female SD rats pre- and post-surgery. Surgery was performed at 7 weeks of age. Rats were randomized into three treatment groups: OVX (n=22); OVX+E2 (n=22); and Sham (n=24). Data are means ± SEM. * Body weights of OVX+E2 group were significantly less than that of the other two groups ($P < 0.05$) at weeks 8–10. ** At weeks 11 and 12, body weights of OVX >Sham >OVX+E2 ($P < 0.05$).

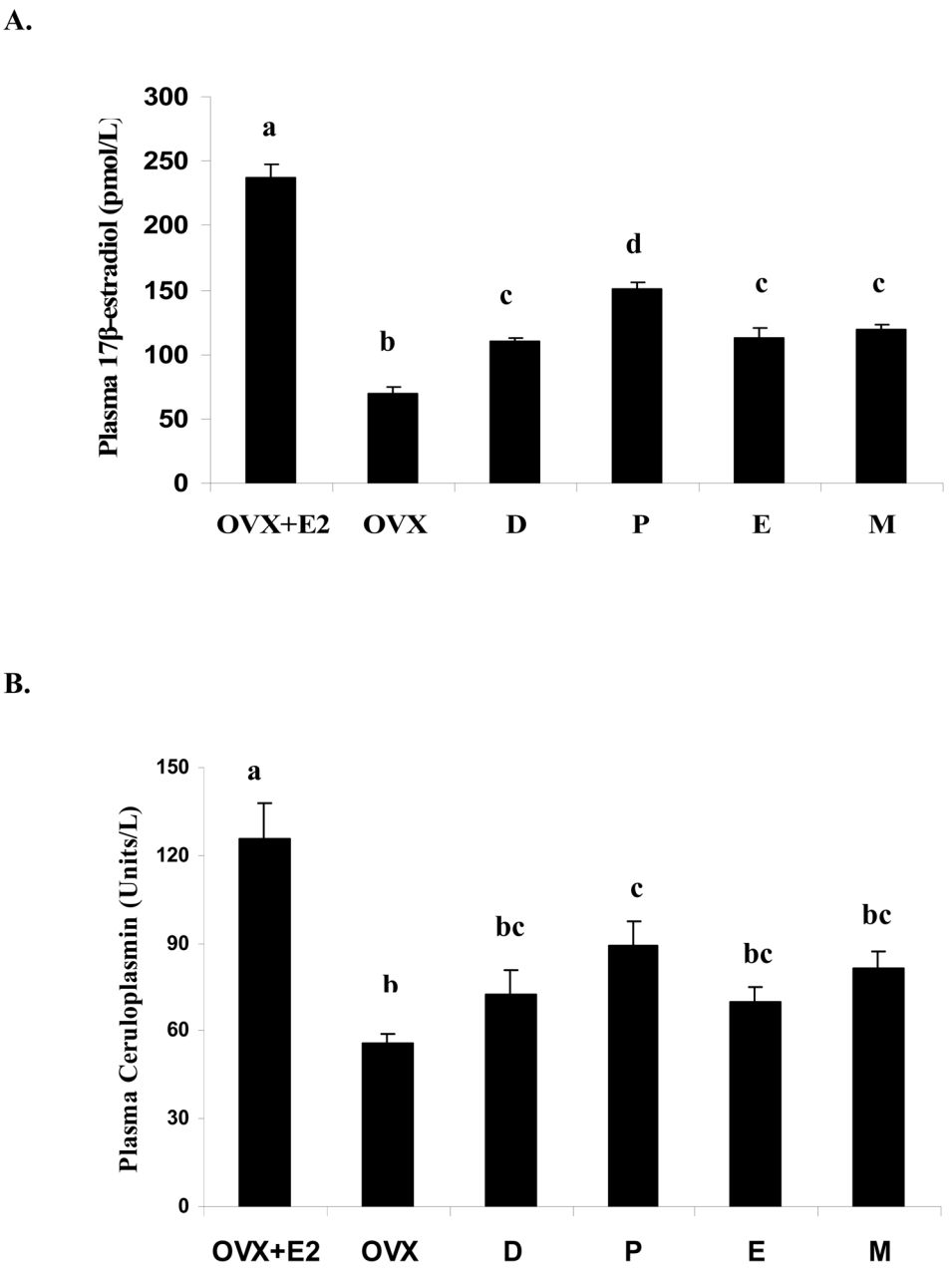

Fig.2.

Plasma concentration of 17β-estradiol (A) and ceruloplasmin activity (B) in treatment groups of female SD rats. The test groups include OVX with estrogen replacement (OVX +E2), OVX with placebo replacement (OVX), and sham-operated with placebo replacement in the diestrus (D), proestrus (P), estrus (E), and metestrus (M) stages. Data are means ± SEM for 6 animals in each group. Bars with different letters differ significantly ($P<0.05$).

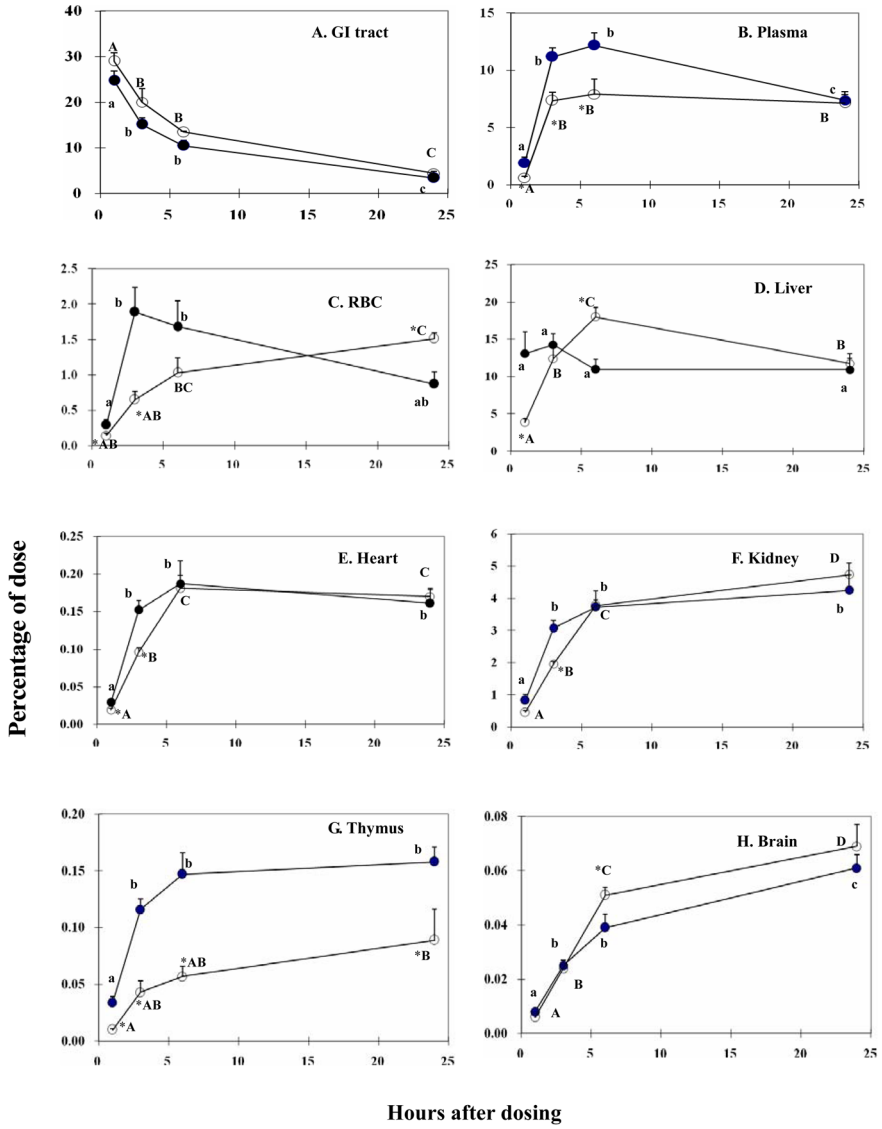

Fig. 3.

^{75}Se activity in tissues at 1, 3, 6, and 24h after gastric administration of 60 μCi $^{75}SeO_3^{2-}$. (OVX, closed circles; OVX+E2, open circles). Panels: A, GI tract; B, plasma; C, RBC; D, liver; E, heart; F, kidney; G, thymus; H, brain. Data are means ± SEM (n=4). Statistically significant difference ($P<0.05$) between OVX group and OVX+E2 group are denoted by presence of an asterisk (*). Within the same treatment (OVX or OVX+E2), means that do not share the same letter are significantly different at $P<0.05$.

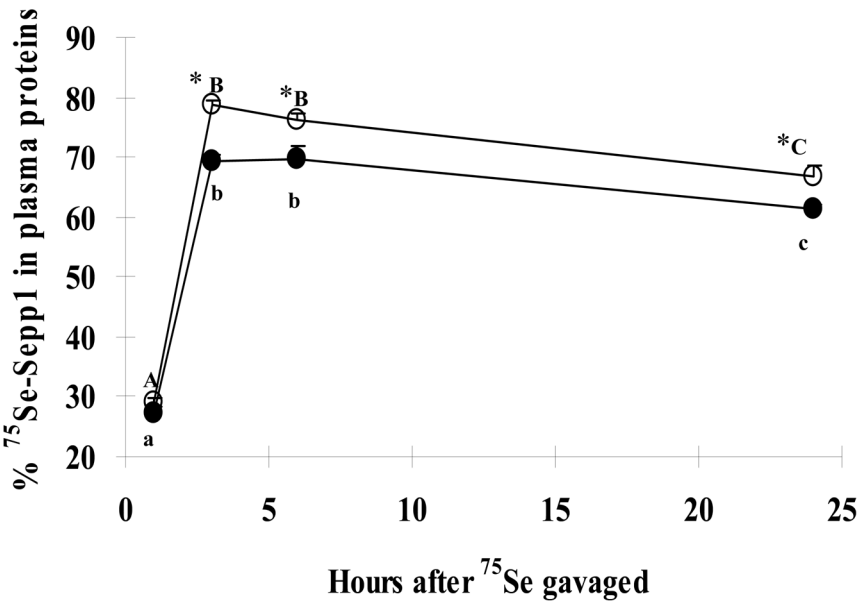

Fig. 4.

^{75}Se activity in plasma Sepp1P at 1, 3, 6, and 24h after administration of $^{75}SeO_3^{2-}$ by gavage (OVX, closed circles; OVX+E2, open circles). Data are means ± SEM (n=4). Statistically significance ($P<0.05$) differences between OVX group and OVX+E2 group at a specific time after dosing are denoted by presence of an asterisk (*). Within the same treatment (OVX or OVX+E2), means that do not share the same letter differ significantly ($P<0.05$).

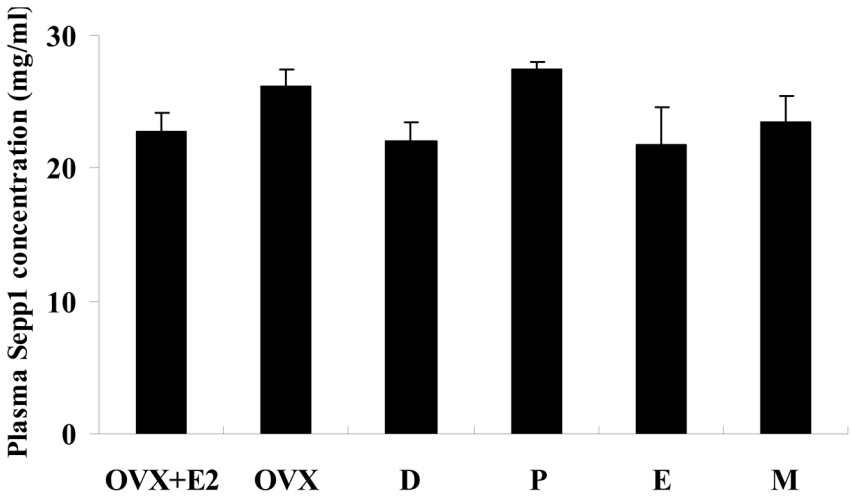

Fig.5.

Plasma Sepp1 concentration in treatment groups of female SD rats. The test groups include OVX with estrogen replacement (OVX+E2), OVX with placebo replacement (OVX), and sham-operated with placebo replacement in the diestrus (D), proestrus (P), estrus (E), and metestrus (M) stages. Data are means ± SEM for 6 animals in each group. No differences in plasma Sepp1 concentrations were observed among groups ($P > 0.05$).

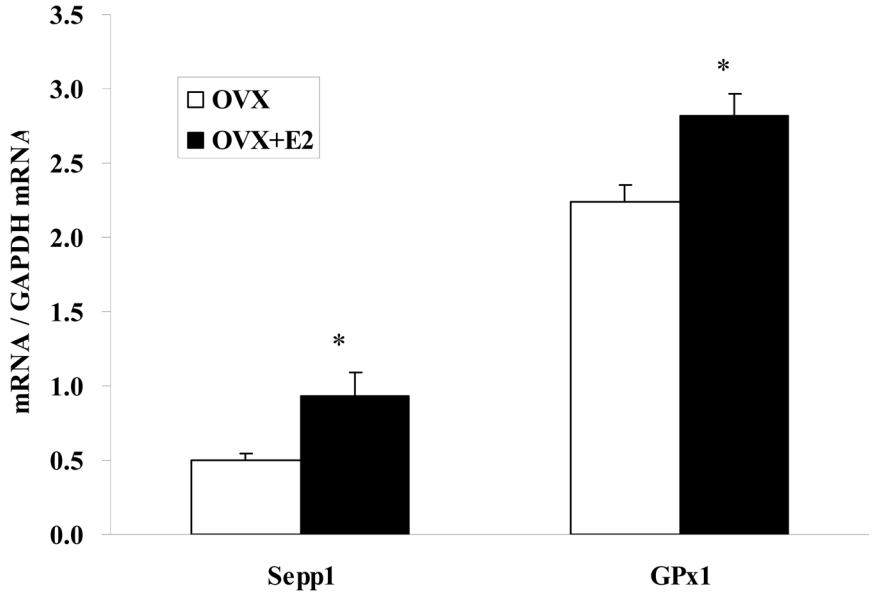

Fig. 6.

Estrogen replacement in OVX rats increases levels of hepatic Sepp1 and GPx1 mRNA. Real-time RT-PCR was carried out with extracted RNA isolated from liver of OVX rats receiving estrogen replacement (OVX+E2) and OVX rats with placebo pellet (OVX). Data are normalized against GAPDH mRNA and presented as means ± SEM for 6 animals in each group. * $P<0.05$ between OVX and OVX+E2.

Table 1

^{75}Se activity in cytosol of organs in female SD rats[1]

Organs	Time after dose	OVX	OVX+E2
Liver	1h	22.4 ± 1.8	11.9 ± 1.7 *
Liver	6h	29.9 ± 2.3	43.1 ± 3.6 *
Heart	1h	0.3 ± 0.1	0.2 ± 0.1 *
Heart	3h	3.7 ± 0.7	1.8 ± 0.2 *
Kidney	3h	37.0 ± 4.6	28.1 ± 2.4 *
Spleen	1h	1.8 ± 0.3	0.7 ± 0.1 *
Thymus	1h	0.6 ± 0.2	0.4 ± 0.1 *
Thymus	3h	3.5 ± 0.6	2.2 ± 0.5 *
Brain	6h	0.3 ± 0.1	0.7 ± 0.1 *

[1] Data are means ± SEM (n=4, dpm/μg protein).

* Statistical significance between OVX group and OVX+E2 group (P<0.05).

Table 2

Effect of estrogen status on selenium concentration in female SD rats[1,2]

	OVX+E2	OVX	Diestrus	Proestrus	Estrous	Metestrus
Plasma, μmol/L	5.61 ± 0.20[a]	3.24 ± 0.08[d]	4.12 ± 0.23[c]	4.84 ± 0.13[b]	4.07 ± 0.15[c]	4.07 ± 0.18[c]
RBC, μmol/L	8.13 ± 0.58[a]	4.58 ± 0.33 [c]	5.21 ± 0.57 [bc]	7.03 ± 0.24[ab]	4.86 ± 0.39[c]	5.09 ± 0.46[c]
Liver, ng/g	1,760 ± 37[a]	1,295 ± 44 [c]	1,505 ± 67 [bc]	1,692 ± 27[ab]	1,482 ± 68[bc]	1,370 ± 67[c]
Kidney, ng/g	1,357 ± 27	1,624 ± 47	1,563 ± 97	1,461 ± 15	1,587 ± 87	1,494 ± 62
Heart, ng/g	423 ± 25	411 ± 19	405 ± 22	450 ± 14	434 ± 21	432 ± 13
Brain, ng/g	441 ± 9[a]	344 ± 9[c]	363 ± 21 [bc]	421 ± 16[ab]	363 ± 21[bc]	366 ± 21[bc]

[1] Test groups include OVX with estrogen replacement (OVX+E2), OVX with placebo replacement (OVX), and sham-operated with placebo replacement in diestrus (D), proestrus (P), estrus (E), and metestrus (M) stages.

[2] Data are means ± SEM for 6 animals in each group and those not sharing a common superscript within each tissue are significantly different ($P<0.05$).

Table 3

Effect of estrogen status on glutathione peroxidase (GPx) activity in female SD rats[1,2]

	OVX+E2	OVX	Diestrus	Proestrus	Estrous	Metestrus
Plasma	146 ± 10[a]	70 ± 6[b]	71 ± 12[b]	119 ± 10[a]	75 ± 11[b]	75 ± 6[b]
RBC	470 ± 12	410 ± 39	427 ± 16	460 ± 42	419 ± 18	417 ± 43
Liver	1,702 ± 60[a]	1,202 ± 90[b]	1,230 ± 66 [b]	1,658 ± 82[a]	1,265 ± 96[b]	1,290 ± 90[b]
Kidney	272 ± 22	364 ± 48	364 ± 43	291 ± 18	316 ± 26	321 ± 41
Heart	289 ± 31	242 ± 27	250 ± 21	328 ± 26	296 ± 24	250 ± 34
Brain	199 ± 9[a]	102 ± 7[c]	133 ± 13 [bc]	169 ± 5[ab]	135 ± 14[bc]	137 ± 17[bc]

[1]Test groups include OVX with estrogen replacement (OVX+E2), OVX with placebo replacement (OVX), sham-operated with placebo replacement in diestrus (D), proestrus (P), estrus (E), and metestrus (M) stages.

[2]Data are means ± SEM (Units/gram protein, Units/gram hemoglobin for RBC) for 6 animals in each group and those not sharing a common superscript within each tissue are significantly different ($P<0.05$).